Physiological, Biochemical and Molecular analysis of static magnetic field induced soybean resistance to anthracnose caused by *Colletotrichum truncatum*

Session 2022-23

A Synopsis Submitted To

Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore

For

DOCTOR OF PHILOSOPHY

In

LIFE SCIENCE

Submitted By

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Enrolment No. - 2205PHDLS13179

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DECLARATION

I, **Niharika Sharma** registered as a research scholar (Enrolment number 2205PHDLS13179) in the faculty of Doctoral Studies & Research, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore, do hereby declare that the synopsis of the research project titled **"Physiological, Biochemical and Molecular analysis of static magnetic field induced soybean resistance to anthracnose caused by *Colletotrichum truncatum*"** has been prepared by me and the work is authentic.

Date:

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2205PHDLS13179

Physiological, Biochemical and Molecular analysis of static magnetic field induced soybean resistance to anthracnose caused by *Colletotrichum truncatum*

Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore

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1. Introduction

According to the World Population Data Sheet for 2020, there will be 9.9 billion people on the planet by the year 2050, up from about 7.8 billion in 2020. The demand for food is expected to increase dramatically as the world population continue to increase at an accelerated rate. The world's agriculture and food industries will be under great pressure than ever before in order to fulfil this demand (Ohta, 2016).

One of the leguminous seed crops with the highest economic importance right now is soybean (*Glycine max*). India is the second-largest producer of soybean in Asia and ranks fifth globally. Rajasthan, Maharashtra, and Madhya Pradesh are the main soybean-producing states (Wilcox, 2016). In accordance to their dry weight, mature, uncooked soybean seeds generally contain 35 to 40% protein, 20% lipids, 9% dietary fibre, and 8.5% moisture (Qin et al., 2022).

The quantity and severity of diseases infecting the soybean crop have grown dramatically along with the global expansion in soybean cultivation area. Pathogens are a key limitation on soybean production affecting yield, protein content, and oil proportion (Sajeesh et al., 2014). In India, *Colletotrichum truncatum*, a significant fungal pathogen, causes soybean anthracnose disease, which results in a yield loss of 16–25% and affects 1.17 million tonnes of soybean annually (Rajput et al., 2021). Under favourable circumstances, this disease has the potential to result in catastrophic losses that can reach up to 100% in regions that produce soybean (Boufleur et al., 2021). Innovative agronomic techniques are needed to safeguard crops in the future due to the threat of resistance and rigorous pesticide regulations.

Chemical or physical treatments applied prior to seeding help seed germination by releasing them from their dormant state. This shields the seeds from pests and viruses and makes it easier for the seeds to grow into a uniform crop stand in the growing area (Sarraf et al., 2020). Based on the usage and subsequent outcome of (magnetic field) MF, one of the physical pre-sowing seed treatments, it has received a lot of attention in agriculture research recently (Araújo et al., 2016). The geomagnetic field is the magnetic field that exists within the earth itself. The magnitude and polarity of the Earth's magnetic field (geomagnetic field) vary depending on where you are on the planet. The magnetic field strength of the Earth's surface varies from 25 to 65 microteslas (μT). Since MF is an energy source, it affects cell metabolism and meristematic cell division (Saletnik, Zaguła, et al., 2022). It has been

discovered that MF therapy for plants and animals is an efficient and developing method to manage illnesses and build tolerance to unfavourable environments (Radhakrishnan, 2019).

It has long been of significant interest to investigate how static magnetic fields (SMFs) affect biological systems. A magnetic field is said to change the structure of the plant cells' membranes, allowing plants to absorb more water and nutrients (Nyakane et al., 2019). In nature, plant growth and yield are impacted by numerous biotic and abiotic stressors.

A number of research have been carried out in order to understand how MF affects seed germination, biochemistry, hormonal changes, plant growth, and yield. Numerous crops, including soybean (*Glycine max*) (Baghel et al., 2016; Kataria et al., 2017; Radhakrishnan, 2019), maize (*Zea mays*) (Afzal et al., 2015; Anand et al., 2012; Florez et al., 2019; Javed et al., 2011; Kataria et al., 2015), moong bean (*Vigna radiata*) (Chen et al., 2011), rice (*Oryza sativa*), wheat (*Triticum aestivum*) (Cakmak et al., 2009; Sen & Alikamanoglu, 2014), and tomato (*Lycopersicum esculentum*) (Selim & El-Nady, 2011) have demonstrated increased productivity and other physiological traits under both non-stressed and stressed conditions (abiotic stesses) after being exposed to magnetic field (Kataria et al., 2017). The method by which plants detect magnetic field and control the signal transduction pathway, yet, is not entirely known. According to research, cryptochromes, which are blue light photoreceptors, are the intermediary for MF sensing and signalling in plants (Ahmad et al., 2007).

All these results, therefore, suggest that the application of MF treatment has a considerable influence on boosting seed germination and seedling vigour in a variety of plant species. The idea of utilizing magneto-priming in improving plant resistance to diverse biotic challenges is encouraged by MF-induced alterations in phytohormone balance, differential protein expression, balanced ROS production, and vigorous plant development under abiotic stresses.

2. Review of literature

Anthracnose is caused by *Colletotrichum* species, which are among the top ten plant pathogenic fungi. They affect more than 3,000 distinct plant species and considerably lower the yield of crops that are important for the economy. The genus is regarded as a model pathogen for biochemical, physiological, and genetic investigations because to its hemibiotrophic lifestyle and the ease with which it may be controlled in research settings (Boufleur et al., 2021; Rogério et al., 2019). At this time, *C. truncatum* is the most prevalent associated species with soybean anthracnose, which has a complex aetiology. Since Korea

provided the first record of a *Colletotrichum glycines* (a synonym of *C. truncatum*) producing soybean anthracnose in 1917, it is possible that the pathogen is endemic to Asia (Boufleur et al., 2021). *C. truncatum* is believed to have originated in China and spread as an invading species to Brazil under the assumption that a pathogenic species' centre of origin should coincide with the host species' centre of genetic diversity (Rogério, 2019). Numerous additional *Colletotrichum* species, including *C. gloeosporioides, C. destructivum, C. chlorophyti, C. coccodes,* etc., have been identified as the disease causative agents in a number of important agricultural crops in the past few years (Boufleur et al., 2021). All development stages of soybean are vulnerable to the anthracnose disease. Common disease signs include pre- and post-emergence damping-off, the development of dark brown cankers on infected cotyledons, veinal necrosis, premature defoliation, and the appearance of brown spots with uneven shapes on stems, pods, and petioles. If the disease progresses, plant death occurs (Rajput et al., 2021; Soto et al., 2020).

Soybean contributes more than 25% of the world's total protein to food and animal feed, making it a significant oilseed crop (Liu et al., 2020). Protease inhibitors, soybean isoflavones, saponins, and proteins are the main soy components that have been demonstrated to have biological action (Chatterjee et al., 2018). From a processing and dietary perspective, it is essential to comprehend the variables that affect the chemical content of soybean and their component structure. Because field trials can't isolate a single environmental element and completely rule out other confounding factors that affect the growth of the soybean crop, the effects of environmental factors are complicated and haven't been fully understood (Medic et al., 2014).

Globally, between 10 and 20 percent of fields are thought to have lost value owing to biotic stressors. To adequately safeguard crops in the future, new agronomic techniques are needed due to the threat of resistance and rigorous pesticide regulations (Natalia et al., 2022). A powerful form of seed invigoration known as seed priming has the power to improve the germination capacity of seeds under a variety of environmental conditions as well as the vigour and germinability of normal seeds (Patel et al., 2022). A broad variety of treatments, some of which are widely known, such as hydropriming and osmopriming, can be used to enhance low-vigor seeds. However, physical techniques such as plasma and magnetic field therapy have demonstrated significant benefits over traditional osmopriming approaches in the realm of seed technology. For a long time, the scientific community has been perplexed and intrigued by the interactions of biological systems with magnetic fields (Barbic, 2019).

According to studies, magnetic treatment can preserve crop output and water quality as well as quantity. There have been numerous studies on the effects of MF or water exposed to permanent magnets on plant growth, including those on the ripening of fruits and vegetables, the growth of farm crops, the presence of bacteria and viruses, the behaviour of animals (Schneider et al., 2023), birds (Wiltschko & Wiltschko, 2019), and aquatic species (Hutchison et al., 2020), among other things.

The seeds that have been magneto-primed had superior germination rates, vigour, seedling biomass, and root growth. Compared to other forms of MFs, the SMF has minimal unstable characteristics, which makes it easier to use it in biological systems. Under SMFs, numerous cellular organelles and components, like mitochondria, cell membranes, proteins, and DNA, alter their electromagnetic behaviour. This has an impact on the cells' physiological and metabolic reactions (Barbic, 2019; Saletnik, Saletnik, et al., 2022). Intriguingly, SMF treatments increase maize antioxidant defences, which appear to increase the plant's ability to withstand abiotic stressors (Anand et al., 2012). Dehydrogenase and protease activities were also discovered to be greater in magneto-primed seeds. The improved seed germination, seedling vigour, and rooting characteristics of seeds treated with SMFs were discovered to be caused by the greater activities of hydrolyzing enzymes. Additionally, it was shown that pre-treating seedlings with MFs made them more resilient to certain abiotic challenges. Under salt stress, magneto-priming enhanced germination and early development characteristics in maize and soybean (Kataria et al., 2017). According to a research by Baghel et al., after receiving SMF treatment, soybean growth, photosynthetic performance, and yield enhanced under water stress circumstances (Baghel et al., 2018). Another study by Kataria et al. revealed that, in contrast to untreated seedlings, soybean seedlings treated with SMF had higher resilience to ambient UV-B stress (Kataria et al., 2020).

To manage anthracnose, a variety of measures have been used, including cultural practises, physical, biological, chemical (fungicide usage), and genetically resistant plant material. *Colletotrichum* is reportedly present in the plant's aerial organs long before anthracnose symptoms manifest. As a result, there is no effect on the disease when fungicide treatments are made after symptoms start to manifest (M. D. Dias et al., 2016). Tebuconazole or thiophenate methyl, which is neither economical nor ecologically safe, is currently sprayed by farmers in India's anthracnose-affected areas to combat the illness (Nataraj et al., 2020). Another factor is that, though a few anthracnose tolerant varieties of soybean are available but farmers at times prefer susceptible varieties owing to more yield, being early, high seed

germinability & longevity, lodging & shattering resistance, bold seed size, and high oil content. Also, due to rapid evolving nature of pathogens, the tolerant varieties become ineffective later. As the disease progresses, plants affected by pathogens often exhibit decreased growth, reduced photosynthetic performance owing to direct damage to the photosynthetic machinery, reduction in the quantity of healthy leaf area, and yield (C. S. Dias et al., 2018). Following a pathogen assault, the host plant's metabolism is also altered, which raises the levels of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radicals (O2•-), hydroxyl radicals (OH•), and singlet oxygen close to the site of the pathogen infection. Antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), peroxidase (POD), and ascorbic acid helps to maintain balancing of ROS (Shine et al., 2012). Superoxide levels in the leaves of field-grown soybean plants were decreased as a result of magnetic field treatment of soybean seeds, claims a report (Baby et al., 2011). According to the findings, magneto-primed cucumber (*Cucumis sativus L.*) seeds showed higher antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione reductase (Bhardwaj et al., 2012).

These studies strongly suggest that pre-treatment of seeds with SMF not only increases the seedlings performance but also aids in mitigating various abiotic stresses. There hasn't been any research done yet on how MF affects plant immunity. Therefore, in this work, we will conduct a thorough analysis of the status of immunity in soybean afflicted by *Colletotrichum truncatum* following magnetic therapy and also utilize it for combating the anthracnose disease.

3. Hypothesis

Understanding how MF affects plants' activities is becoming more and more important since, according to recent studies, plants may quickly alter their gene expression, biochemistry, and phenotypic alterations in response to MF. This method is a non-invasive technique that is friendly to organic farming, which is a quality that is highly valued in contemporary agriculture. In the past, it has been discovered that pre-treating soybean seeds with static magnetic field improves plant development, protein expression, ROS homeostasis, and photosynthetic metrics when compared to untreated ones in the presence of diverse abiotic challenges such heat, cadmium, salinity, drought, and ambient UV-B stress. To our knowledge, no research to analyse soybean immunity against biotic stress following magnetic field therapy has been conducted. Considering the strong impact of static magnetic field

treatment, the objective of this investigation is to determine whether pre-treatment of soybean seeds with a static magnetic field reduces the negative impacts of the anthracnose disease on biochemical, physiological, and molecular traits in soybean plants.

4. Objectives

4.1. Selection of appropriate *Colletotrichum truncatum* susceptible and tolerant soybean varieties.

4.2. Magnetic field pre-treatment to seeds and biochemical assay to study the impact of static magnetic field treatment on soybean resistance against anthracnose disease in susceptible and tolerant varieties of soybean.

4.3. Differential transcriptome profiling of susceptible and tolerant soybean varieties post static magnetic field treatment for the identification of key defense regulators.

4.4. Pathogenicity assay to unravel the impact of MF treatment on enhancing the tolerance of susceptible soybean variety as compared to that of control plants.

5. Material and methods

5.1. Selection of *C. truncatum* susceptible and tolerant soybean variety: Extensive and systematic survey will be conducted in various soybean growing regions to pinpoint specific anthracnose susceptible varieties of soybean that are popular and highly accepted by the farmers. Likewise, soybean varieties tolerant to *C. truncatum* infection will be selected, which shows resistance to *C. truncatum* but is not popular among farmers due to undesirable traits such as late maturing, small seed size, low oil content; and lodging and shattering prone.

5.2. Procurement of plant and fungal material: The breeder seeds of soybean susceptible varieties and tolerant varieties will be collected from ICARIISR (full form), Indore, India. Seeds of uniform size and shape without visible defects, and malformation will be selected and stored. *Colletotrichum truncatum* culture will be procured from ICAR-IISR, Indore, India. *C. truncatum* mycelial colony will be propagated on potato dextrose agar (PDA) medium. The fungal cultures will be incubated at 28℃ with a 12 h day/night cycle under cool white fluorescent lamps.

Every 7–10 days, fungal isolates will be transferred to fresh PDA plates. The authenticity of the cultures will be confirmed by colony morphology and microscopy.

5.3. Magnetic field generator: The soybean seeds will be treated using an electromagnetic field generator called "EMU-75" that was constructed and installed in the Department of Electrical and Electronics Engineering (SVVV-Indore) with a separation of five cm among pole components **(Figure 1)**. A digitized gauss analyzer based on the Hall Effect, brand DGM-102 (Scientific Equipment Roorkee), will be utilized for measuring the field strength produced in the pole gap.

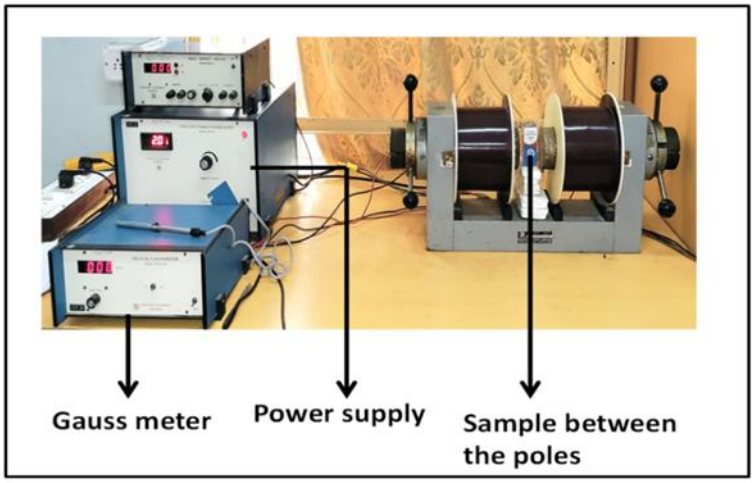

Figure 1: Electromagnetic field generator (pole gap of 5 cm).

5.4. Magnetic treatment: Soybean seeds will be exposed to pre-standardized magnetic field of 200 mT in a cylindrical shaped sample holder, made from a non-magnetic thin cardboard sheet. One hour of treatment was given to 100 grown, healthy, and visually sound seeds that were housed in a plastic container and put between the poles of an electromagnet.

5.5. Plant growth, Infection, Biochemical assay, and Transcriptome profiling

5.5.1. Planting: Soybean seeds (magnetic field treated and control) of both the susceptible variety and tolerant varieties will be sown in plastic pots (filled with mixture of coarse sand and black soil) and kept in polyhouse. Any required

watering will be given to plants in addition to actual physical weed control. The experiments will be conducted with three replicates for each treatment. The experiments will be conducted in three replicates for each treatment.

5.5.2. Inoculum preparation: *C. truncatum* culture will be grown on potato dextrose agar (PDA) medium with alternating 12 h of light and dark condition at 28 ± 1°C for 7 to 10 days to facilitate spore production. Conidia will be harvested from the plates by adding 10 ml of sterile distilled water per plate. The surface will be brushed using a plastic disposable loop. Then, spores mixture will be filtered through a double layer of cheesecloth and diluted with water to a final concentration of 2.5 X 10^6 conidia per milliliter.

5.5.3. Plant infection: The conidial suspension of C. truncatum will be sprayed over the adaxial and abaxial leaves of each soybean plant after the plants have grown for 21 days (V4 growth stage).

5.5.4. Biochemical assay: The trifoliate leaf of plants from each replication and treatment will be collected. Leaf samples will be used for determination of protein by Lowry method. Peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), polyphenyl oxidase (PPO), Phenylalanine Ammonia-Lyase (PAL) activities of trifoliate leaf samples will be determined.

5.5.5. RNA-Seq of soybean plant samples: RNA-Seq will be performed in triplicates of the following four soybean plant samples using Illumina sequencing platform: (a) Susceptible soybean variety (as control); (b) MF pre-treated susceptible soybean variety; (c) Tolerant soybean variety (as control); (d) MF pre-treated tolerant soybean variety

Analyses of obtained RNA-Seq data: Data obtained will be analyzed for the presence of differential expressed genes (DEGs) in the MF treated versus control samples.

5.6. Pathogenicity assay: The MF treated susceptible and tolerant soybean varieties along with the untreated control seeds will be grown in the field and infected at

seedling stage with *C. truncatum* for pathogenicity assay. A highly virulent and prevalent isolate of *C. truncatum* will be selected, purified by single spore culture and maintained on PDA medium. This isolate will be used to challenge the soybean plants in order to study the disease severity. After inoculation with mycelial suspensions, the disease severity will be recorded at R6 stage, in terms of percent pod infection using 1–5 scale. This pathogenicity assay will unravel the impact of SMF treatment on resistance of susceptible soybean variety as compared to that of control plants. A comparative statistical analysis of anthracnose disease symptoms will also be carried out between SMF treated susceptible soybean variety and untreated tolerant soybean variety to investigate if SMF treatment enhances the disease resistance in susceptible soybean variety comparable to that of tolerant variety.

5.7. Statistical analysis: One-way analysis of variance (ANOVA) will be used to examine all data, which will be presented as means ± (SD) standard deviations.

6. Future Outcomes

MF induced changes in phytohormone balance, differential protein expression, balanced ROS generation and robust plant growth under abiotic stresses encourages the possibility of role of magneto-priming in enhancing the resistance of plants during various biotic stresses (like fungal infection). Looking at the economic importance and severity of the anthracnose disease, this study will be aimed at investigation of impacts of SMF on soybean immunity against the disease. With the help of this study, it will be possible to demonstrate that physical control techniques like magnetic field therapy may have potential for preventing anthracnose disease and stimulating a plant's immune system while also being distinguished by their lack of side effects compared to those brought on by commonly used chemical methods such as fungicides.

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